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The Ability of Namnam (*Cynometra cauliflora*) Leafs Extract as Antidiabetic Agent Through α–Glucosidase Inhibition on Several Extraction Stages

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Abstract

The prevalence of patients with Diabetes Mellitus (DM) is increasing dramatically worldwide. One therapeutic approach to treat diabetes by inhibiting the absorption of glucose through inhibiting α-glucosidase. Plant that is potential for this benefit is namnam (*Cynometra cauliflora*). α-glucosidase inhibitory activity of namnam leafs extract (*Cynometra cauliflora*) can be tested at various stages of extraction such as maceration with methanol, liquids extraction (solvent: n-hexane, ethyl acetate and n-butanol), and column chromatography. α-glucosidase inhibitory activity was tested in vitro using a p-nitrophenyl- α-D-glucopyranoside (PNPG) as a substrate.

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The analysis showed that the IC_{50} value at maceration stage with methanol is 5.58 ± 2.0 µg/mL, while the IC_{50} value at liquid-liquid extraction are 1.84 ± 0.9 µg/mL (n-butanol), 16.22 ± 1.03 µg/mL (n-hexane), 21.92 ± 0.23 µg/mL (ethyl acetate), and 37.74 ± 2.74 µg / mL (water). Column chromatography stage of fraction I and fraction II showed LC_{50} values of 54.30 ± 2.13 µg / mL and 90.16 ± 0.72 µg / mL, respectively. Phytochemical analysis result also showed that at each stage contains saponins, steroid or triterpenoid, phenolic, flavonoids, tannins, and quinones. Thus; the leafs extract of Namnam (*Cynometra cauliflora*) has potency as an antidiabetic agent through the inhibition of α-glucosidase.

Keywords: α-glucosidase; Antidiabetic; *Cynometra cauliflora*; Extraction; Namnam .

1. Introduction

The prevalence of diabetes has arisen dramatically worldwide [1]. It is estimated that the prevalence will grow up to be 380 million in 2025 [2]. Approximately 90% of these patients are type 2 diabetes mellitus (T2DM) [3] and only 5% are Type I Diabetes Mellitus (T1DM) [4]. WHO mentions that Indonesia at 4^{th}rank for country whose people suffer diabetic in the world following to India, China and the United States. This disease is very heterogeneous because it is caused by the interaction of various complex factors such as metabolism, environment and genetic [5].

T2DM is provoked by an intrusion in the ability of insulin in stimulating blood glucose by cells [6]. One therapeutic approach for treating diabetes is the hamper of absorbing glucose by inhibiting α -glucosidase. Alpha-glucosidase is an enzyme located on the surface of the brush border of intestinal cells which plays an important role in the process of digesting oligosaccharides into mono saccharides in order to being easily absorbed by the intestine [7].

Various attempts were made to obtain inhibitors for α –glucosidase, which were effective and safe, from natural products such as guava leafs [8], fucoidan from *Fucus vesiculosus* and *Ascophyllum nodosum* [9], trilobatin from *Lithocarpus* polystachyus [10], *Cichorium glandulosum* seed [11], tea (*Camellia sinensis* L) [12], and soybean extracts [13].

Another potential plant for this purpose is Namnam (*Cynometra cauliflora*). Results of previous studies show that the stem, leafs and young leafs of Namnam plant contain bioactive components such as terpenoids, tannins, saponins, flavonoids and cardiac glycosides [14]. Ado and his colleagues have also reported that the extract of ethyl acetate (EtOAc) and n- butanolina Namnam leafs resulted from liquid partition has potential inhibitory activity on α – glucosidase enzyme. The IC50 value of the ethyl acetate (EtOAc) and n-butanol extracts are namely 30mg/mL and 44 mg/mL [15]. Sumarlin and his colleagues also shows that the methanol extract of the leafs of this plant contains flavonoids [16].

According to a research by Tadera and his colleagues in-vitrolly, it suggests that the flavonoid compound is a compound that is able to inhibit α – glucosidase [17]. This is supported by Unnikrishnan and his colleagues (2014) which explains that flavonoids act as an antidiabetic agent [18]. Therefore,Namnam (*Cynometra cauliflora*) is also thought to have the ability as α - glucosidase inhibitors and potential as an antidiabetic agent.

2. Materials and Method

The test materials used in this study are the Namnam (*Cynometracauliflora*) leafs obtained from *Desa Cintaratu, Kecamatan Parigi, Kabupaten Pangandaran, West Java-Indonesia* and has been identified by the Bogor-based Research Center for Biology, Indonesian Institute of Sciences as *Cynometra cauliflora* L, phytochemical test reagent, a standard solution of quercetin, methanol (p.a), n-butanol (p.a), n – hexane (p.a), ethyl acetate(p.a), DMSO (*Dimethyl sulfoxide)*, buffer phosphate pH 7, acetone, petroleum ether, p-nitrophenyl-α-D-glucopyranoside (PNPG) 20 mM, α - glucosidase derived from *Saccharomyces cereviseae*, Na_2CO_3 0,2 M, aluminum foil, aquades, phosphate buffer solution20 mM (pH 6,9), and sillica gel$G_{60}F_{254}$.

2.1. Preparation of Samples [15]

Namnam (*Cynometra cauliflora* L.) leave were washed, sorted and dried by the sun for 30 hours until 9-10% moist. The dried leafs were then grinded by a blender in order to obtaining smooth powder. Namnam leafs powder weighing 100 gram was soaked with 100 mL of methanol and macerated for 24 hours. Following to that step, the macerated powder was filtered with Whatman filter paper no.4 in order to obtainingthe first filtrate. Namnam leafs residue was re-macerated with methanol solvent for 9 hours to get the second filtrate. The filtrate was evaporated by rotary evaporator with 49°C temperature to obtain viscous extract. The extraction process required to be conducted five times before being ready to be brought to the next process.

2.2. Phytochemical Studies

Phytochemical analyses of the extract were performed according to the methods of Harborne. The extract was screened for the presence of alkaloids, saponins, tannins, flavonoids, quinons and steroids/triterpenoids.

2.3. Liquids Extraction [15]

The method of liquids extraction was done using separating funnel. Before being partitioned, dried methanol extract was firstly dispersed with warm water to facilitate the solubility, then it was fractionated with n- hexane, ethyl acetate, n-butanol and water until two phases were formed and separated. Partitioning was conducted repeatedly until the above methanol phase were looked clear which indicated that the secondary metabolite compound on the sample had completely dissolved into the solvent. Each phase which was obtained is evaporated with a vacuum rotary evaporator at 70°C, and then dried in an oven at 50°C to obtain dry extract.

2.4. Chromatography Columns [19]

The most active extract from inhibitory activity test (α -glucosidase) were weighed, before being made as a test solution which would be separated from column chromatography by a stationary phase in the form of silica gel$G_{60}F_{254}$size of 0.2-0.5 mm. Eluent which was used must be able to separate the sample well. Therefore, it was elected based on the best separation pattern on a thin layer chromatography. The procedures of column chromatography are as follows:

a. 1.5 grams samples or viscous extracts were dissolved by a solvent, and as much as 46 grams silica gel was added and stirred until a thick extract turned dry.
b. Dried silica gel was added to the column and compacted.
c. Solvent was added into the column followed by dried extracts.
d. Acetone eluent: petroleum ether = 70:30, 80:20, 90:10, 100 : 0 were added.

The result of Thin Layer Chromatography (TLC) that had similar Rf were collected in the same fraction, namely Fraction I (FI) and Fraction II (FII). The result of maceration by methanol and fractionation from several solvents such as n-hexane, ethyl acetate and n-butanol, column chromatography (FI and FII) was used for the inhibition activity of α –glucosidase test.

2.5. α-Glukosidase Inhibition Activity Test [20]

Each sample of the methanol extracts (resulted from maceration), n - hexane, ethyl acetate, n - butanol, and Namnam leafs water were weighed as much as 2 mg and dissolved in 100 mL DMSO. Then, it was made standard solution with various concentrations of 100; 50; 25; and 10 ppm. 5 mL samples were inserted into the tube and added with 495 mL of phosphate buffer pH 7 and 250 mL of 20 m Mp-nitrophenyl-α-D-glucopyranoside (PNPG). After becoming homogeneous, the solution was pre-incubated for 5 minutes at the temperature of 37ºC, then added with 250 mL of α - glucosidase and incubated for 15 minutes.

The reaction was stopped by the addition of 1 mL of 0.2 M Na_2CO_3. The amount of resulted p-nitrophenol was measured at a wave length of 400 nm using a UV - Vis Spectrophotometer.

The calculation of % of the inhibition for each sample and IC_{50} concentrations in each sample according to the formula is shown below:

$$\% \text{ inhibition} = \frac{C-S}{C} \times 100\%$$

Where: S= absorbance of the sample (s)

C = absorbance of control(DMSO) (c)

IC_{50} can be calculated by using linear regression equations, sample concentration as the x-axis and % inhibition as the y-axis. From equation: y = a + bx IC_{50} values can be calculated by using formula:

$$IC_{50} = \frac{50-a}{b}$$

3. Results

The result of phytochemical test on Namnam leafs extracts shows that it contains secondary metabolites such as saponins, steroid or triterpenoid, phenolics, flavonoids, tannins, and quinones (Table 1).

Table1: Chemical constituents of various solvent and extractions of *Cynometra cauliflora*

Chemical constituens	Type of Solvent for Extraction				
	Methanol*	n-hexane**	ethyl acetate**	n-butanol**	Water**
Saponins	+	+	+	+	+
Steroids/triterpenoids	+	+	+	+	+
Flavonoids	+	+	+	+	-
Tannins	+	+	+	+	+
Quinons	+	+	+	+	+
Alkaloids	-	-	-	-	-

(+) = Detected, (-) = Not Detected. * = Maceration results [16]** = Liquids Extraction

3.1. Total Phenolic Contents

The content of total phenolic (Table 2) in each extract is expressed as gallic acid equivalents (GAE). GAE is a common reference for measuring phenolic compounds contained in a material [21]. The determination result of total phenolic (Table 2) is acquired that the highest levels of total phenolic is possessed by methanol extract at maceration stage, followed by ethyl acetate extract and butanol extract.

Table 2: Total phenolic content of *Cynometra cauliflora* by the different solvents and methods extractions

Extract/Fraction	Total phenolic content (mg GAE/g sample)
Methanol	$210{,}94^{a} \pm 1{,}06$
n-hexane	$9{,}81^{b} \pm 0{,}09$
Ethyl acetate	$170{,}20^{c} \pm 0{,}07$
n-butanol	$52{,}47^{d} \pm 0{,}26$
Water	$9{,}3^{e} \pm 0{,}26$

Figures followed by different letters indicate significant differences at the level of advanced test of Duncan 5 % significance level of 95 % ($P < 0.05$)

3.2. α-Glucosidase Inhibition Assays

The test of inhibition activity of α -glucosidase is based on in-vitro enzymatic reactions using p- nitrophenyl - α - D – glucopyranoside (Table 3) as the substrate and quercetin as the positive control [22].

Table 3: The inhibitory activity of the *Cynometra cauliflora*leafs extract on α-glucosidase by different solvents and methods extractions

Extract/Fraction	Ulangan	% Inhibition			IC_{50} (μg/mL)
		10 ppm	25 ppm	50 ppm	
Methanol	1	65,59	95,10	99,14	5,59[a] ± 1,95
	2	55,71	96,09	99,37	
n-hexane	1	13,33	90,97	95,58	16,22[b] ± 1,03
	2	20,32	92,78	96,10	
Ethyl acetate	1	8,91	56,27	92,40	21,92[c]± 0,23
	2	8,01	60,33	91,72	
n-butanol	1	77,92	96,14	100	1,84[d]± 0,88
	2	72,49	95,37	100	
Water	1	3,26	13,28	69,69	37,74[e] ± 2,74
	2	3,27	19,82	72,62	
	1	34,29	55,96	83,99	16,05[f] ± 1,27
Quercetin (standart)	2	38,73	63,88	82,68	

Where: a = Not significantly different at advance test level Duncan 5 % significance level of 95 % ($P < 0.05$)

3.3. The Inhibitory Activity on α-Glucosidaseby Fraction Chromatography Columns

Table 4: The inhibitory activity of *Cynometra cauliflora* leafs extract on α-glucosidase by Fraction Chromatography columns

Sample	Repeated	% Inhibitions				IC_{50} (μg/mL)
		10 ppm	25 ppm	50 ppm	100 ppm	
	1	7,4988	20,2983	55,0675	84,1756	54,30 ± 2,13
FI	2	6,6445	23,6760	49,7411	94,3040	
	1	3,5990	8,3560	26,7531	56,3621	90,16 ± 0,72
FII	2	5,1199	14,1715	25,2668	55,9386	

Description: FI= FractionI, FII= FractionII

4. Discussion

Secondary metabolite is an active component that has potential as an antidiabetic compound. The analysis also shows that in all of the solvents and extraction stages, several photochemical compounds can be detected and measurable. Aziz and Iqbal, (2013) have tested the phytochemicals C. *cauliflora* which shows the presence of tannins, saponins and flavonoids [14]. However, alkaloids are undetectable in all stages and flavonoids are not detected in the water phase.

The ability of Namnam leafs extract (*Cynometra cauliflora*) inhibiting the activity of α - glucosidase is thought to be caused by the presence of phenolic compounds in this plant. The existence of these compounds has also been shown by the phytochemical test (Table 1) and total phenolic test (Table 2). Other studies also support a role of this phenolic compound such as insweet potato [23], Berry fruit [24], *Psidium guava* dan *Syzygium cumini* polyphenols [25] as an inhibitor of α- glucosidase.

The differences in total phenolic content of each extract are influenced by compounds distribution based on the polar characteristics of the solvent. The solvent which is more polar have better ability in the extraction of phenolic compounds compared to the solvent that has lower polarity such as n- hexane. Some phenolic compounds found in plants have been reported to have various biological activities [26].

The antidiabetic test result shows that the percent of inhibition in Namnam (*Cynometra cauliflora*)leafs extract toward α –glucosidase is increasing in line with the increment of the concentration of Namnam (*Cynometra cauliflora*) leafs extract. *Cynometra cauliflora* extract with 25 ppm and 50 ppm concentration have higher inhibition levels compared to the 10 ppm concentration (Table 3). The increment of the inhibition percentage occurs for high concentrations, there are more active components in the samples which have the ability to inhibit the activity of α - glucosidase. The existence of the active components as α -glucosidase inhibitors has been raised by Qaisar and his colleagues [27] in 2014. Additionally, Kakoos [28] has also demonstrated that the inhibition on α - glucosidase is dependent on the concentration of the methanol extract of *Centaurea calcipatra* which shows that the higher concentration is, the higher percent inhibition is.

The aspects of the inhibition are also seen from IC_{50} (Inhibitory Concentration 50). The result $IC_{50,}$ shows that the extraction process and usage of different solvents are various. As the IC_{50} is lower, so the ability of the plant extract in inhibiting α –glucosidase gets higher. Such result shows that the level of inhibition on α -glucosidase increases from maceration process to the process of liquids extraction using n-butanol, but lower it gets lower in n-hexane and ethyl acetate (Table 3). It is also showed that the methanol extract (maceration) and butanol extract (liquids extraction) are higher than the standard quercetin. The result also shows that the liquid extraction process is able to separate the contained chemical compounds so it obtains the active compound which is thought to be able to inhibit the α–glucosidase. Other plants that are in the same genus *Fabaceae* have also been studied with IC_{50}, such as *Cassia alata* L. (leafs) 50.54, *Gycine max* Merr (seeds) 6,645.97, *Phaseolus vulgaris* L. (seeds) 4.83 (ppm) [29].

The analysis result of samples of Namnam (*Cynometra cauliflora*) leafs extraction through chromatography

shows a decrement in activity compared to the maceration process (methanol extract) and liquid fractionation process (n-butanol extract). This is demonstrated by the increment in the IC_{50} value at each sample of fraction I (FI) and Fraction II (FII). Nevertheless, every concentration increment on Namnam (*Cynometra cauliflora*) leafs extract, the percent inhibition will get higher (Table 4).

The decreasing inhibitory activity toward α -glucosidase compared to butanol crude extract is thought to be caused by two things: (1) Active compounds which play a role as inhibitors of the FI and FII are lost during the separation process using column chromatography. This phenomenon is elaborated by the existence of the decreasing activity of anti-candida due to the changes in the chemical components of essential oils, especially the loss of the active compound which is responsible for the activity during the extraction process [30], (2) some compounds that have roles asagents for inhibiting α -glucosidase in crude n - butanol extract are thought to be synergistic so that they give stronger inhibition energy. The synergistic compounds are allegedly able to either change or lose when they undergo the next extraction process.

According to the research by Wang and his colleagues [8] the combination of two inhibitors compounds namely *quercetin - myricetin*, *hyperin – avicularin*, and *quercetin-kaempfer on* that are isolated from guava leafs shows significant improvement and performs the synergistic work toward α-glucosidase at the same time. Such increment of the activity is acquired by differentiating the IC_{50}value of the compound inhibitor combination and the IC_{50} value of each single compound. For instance, the IC_{50} value myricetin - quercetin (2.0 mM) is lower than the IC_{50} value of single compound myricetin (3.0 mM) or quercetin (3.5 mM) (Table 5).

Table 5: The possible synergistic activities of the isolated flavonoid compounds toward α-glucosidase [8]

Flavonoids	Sucrase	Maltase	Interaction
	IC_{50} (mM)	IC_{50} (mM)	
Quercetin	3.5 ± 0.3	4.8 ± 0.4	Synergistic
Myricetin	3.0 ± 0.1	4.1 ± 0.2	
Quercetin+myricetin	2.0 ± 0.2	3.2 ± 0.4	
Hisperin	7.5 ± 0.8	7.8 ± 0.6	Synergistic
Avicularin	6.5 ± 0.7	7.6 ± 0.2	
Hisperin+avicularin	4.5 ± 0.5	5.0 ± 0.3	
Kaempferol	5.2 ± 0.4	5.6 ± 0.1	Synergistic
Quercetin	3.5 ± 0.3	4.1 ± 0.2	
Kaempferol+Quercetin	2.6 ± 0.6	4.2 ± 0.1	

This prediction is also strengthened by the analysis result of phenolic compound (Table 2) that the height of phenolic compound is not linear to the IC_{50} value, which means that the inhibition on α -glucosidase is not only

caused by the presence of phenolic compound, but also it is caused by other factors. Alagesan and his colleagues also found that *Psidium guajava* (leafs) with a 250 mg/gphenol concentration and *Syzygium cumini* (seed) 180 mg/g have LC_{50} of each 10 ± 0.04 mg/mL and 8 ± 0.17 mg/mL [31], but how the mechanism and what compound that practically play a role in compound resulted from the extraction of Namnam (*Cynometra cauliflora*) leafs require further research.

Nevertheless, this result has clearly indicated that the compound components contained in FI and FII are potential to be α –glucosidase inhibitor. The inhibition on α -glucosidase is an approach in the medication of type 2 diabetes mellitus. This approach is conducted through inhibiting postprandial blood glucose that goes into blood circulation. This activity is predicted to be executed by Namnam (*Cynometra cauliflora*) leafs extract, so it has potential as an anti-diabetic agent, especially type 2 diabetes mellitus.

5. Conclusion

According to the study on Namnam (*Cynometra cauliflora*) leafs extract,with several stages of extraction,it indicates that:

1. At the stage of maceration using methanol IC_{50} amounted5.59ug/mL that undergo an increment in the liquid fractionation with n- butanol solvent IC_{50} amounted 5.58mcg/mL. However, following to column chromatography, it experiences a decreasing activity with an increment of IC_{50} value in Fraction I and Fraction II, respectively 54.30 mg/mL and 90.16 mg/mL.
2. Namnam (*Cynometra cauliflora*) leafs extract has potential as an anti-diabetic agent through the inhibition process on α - glucosidase

References

[1] J. Gorelick, A. Kitron, S. Pen, T. Rosenzweig and Madar Z. “Anti-diabetic activity of Chiliadenusi phionoides”. J Ethnopharmacol, vol. 137, pp. 1245–1249, Oct. 2011

[2] Silink, M.http://www.eatlas.idf.org/newsc269.html, accessed March 2015

[3] American Diabetes Association, “Diagnosis and classification of diabetes mellitus, Diabetes Care”. vol. 28, pp. S37–S42, 2005.

[4] [IDF] International Diabetes Federation, Diabetes Atlas Edisi ke-6. 2013, Brussels: International Diabetes Federation.

[5] S. Kadan, B. Saad , Y. Sasson and H. Zaid. “In vitro evaluations of cytotoxicity of eight antidiabetic medicinal plants and their effect on GLUT4 translocation”. Evid. Based Complement. Altern. Med, 549345, Mar. 2013

[6] C.M. Orme and Bogan J.S. “Sorting out diabetes”. Science, vol. 324, pp. 1155–1156, May 2009.

[7] S.D. Kim. “α-Glucosidase inhibitor from Buthus martensi Karsch”. Food Chem, vol. 136, pp. 297–300, Jan. 2013.

[8] H. Wang, Y.J. Du and H.C. Song. “α–Glukosidase and α–Amylase Inhibitory Activities of Guava Leaves”. Food Chem, vol. 123, pp. 6-13, Nov. 2010

[9] K.T. Kim, L.E. Rioux and S.L. Turgeon. “Alpha-amylase and alpha-glucosidase inhibition is differentially modulated by fucoidan obtained from Fucus vesiculosus and Ascophyllum nodosum”. Phytochemistry, vol. 98, pp. 27–33, Feb. 2014

[10] H.Q. Dong, M. Li, F. Zhu, F.L. Liu and J.B. Huang. “Inhibitory potential of trilobatin from Lithocarpus polystachyus Rehd against α-glucosidase and α-amylase linked to type 2 diabetes”. Food Chem, vol. 130, pp. 261–266, Jan. 2012.

[11] X. Yao, L. Zhu, Y. Chen, J. Tian and Y.Wang. “In vivo and in vitro antioxidant activity and α-glucosidase, α-amylase inhibitory effects of flavonoids from Cichorium glandulosum seeds”. Food chem, vol. 139, pp. 59-66, Aug. 2013.

[12] Y. Wang, S. Huang, S. Shao, L. Qian, and P. Xu. “Studies on bioactivities of tea (Camellia sinensis L.) fruit peel extracts: Antioxidant activity and inhibitory potential against α-glucosidase and α-amylase in vitro”. Industrial Crops and Products, vol. 37, pp. 520– 526, May 2012.

[13] A.O. Ademiluyi and G. Oboh. “Soybean phenolic-rich extracts inhibit key-enzymes linked to type 2 diabetes (α-amylase and α-glucosidase) and hypertension (angiotensin I converting enzyme) in vitro”. Exp Toxicol Pathol, vol. 65, pp. 305–309, Mar. 2013.

[14] A.F.A. Aziz and M. Iqbal. “Antioxidant Activity and Phytochemical Composition of Cynometra cauliflora”. Journal of Experimental Integrative Medicine, vol. 3, pp. 337-341, Aug. 2013

[15] M.A. Ado, F. Abas, I.S. Ismail, H.M. Ghazali and K. Shaari. “Chemical Profile and Antiacetylcholinesterase, Antityrosinase, Antioxidant and α-Glucosidase Inhibitory Activity of Cynometra cauliflora L. Leaves”. J Sci Food Agric, vol. 95, pp. 635-642, Feb. 2015.

[16] L. Sumarlin, A Suprayogi, M Rahminiwati, A Tjachja and D Sukandar. “Bioactivity of Methanol Extract of Namnam Leafs in Combination with Trigona Honey”. Jurnal Teknologi dan Industri Pangan, vol. 26, pp. 144-154, Dec. 2015.

[17] K. Tadera, Y. Minami, K. Takamatsu and T. Matsuoka. “Inhibition of α-Glukosidase and α-Amylase by Flavonoids”. J Nutr Sci Vitaminol, vol. 52, pp. 149-153, Apr. 2006.

[18] P.S. Unnikrishnan, K. Suthindhiran and M.A. Jayasri. “Inhibitory Potential of Turbinaria ornata against Key Metabolic Enzymes Linked to Diabetes”. BioMed Research International, Vol. 2014,

Article ID 783895, 10 pages, Jun. 2014,

[19] G.R.F. Tchouya, G.D.N. Obiang, E.A. Nantia and J. Lebibi. "Phytoche, Antimicrobial and Antioxidant Evaluation of The Stem Bark of Thomander siahensii (Acanthaceae)". Jornal of Pharmacognosy and Phytochemistry, vol. 3, pp. 190-195, Oct. 2014,

[20] Y.M. Kim, Y.K. Jeon, M.H. Wang, W.Y. Lee, and H.L. Rhee. "Inhibitory Effect of Pine Extract on α-Glucosidase Activity and Postprandial Hyperglycemia". Nutrition, vol. 21, pp. 756-761, Jun. 2005

[21] M. Mongkolsilp, I. Pongbupakit, S.L. Nittaya and W. Sitthithaworn. "Radical Scavenging Activity and Total Phenolic Content of Medicinal Plants Used In Primary Health Care". SWU J Pharm Sci, vol. 9, pp. 32-35, Apr. 2014.

[22] R.T. Dewi, S. Thacibana and A. Darmawan. "Effect of α-Glukosidase Inhibition and Antioxidant Activities of Butyrolactone Derivatives from Aspergillus Terreus MC75". Medical Chemistry Research, vol. 23, pp. 454-460, Jan. 2014.

[23] T. Matsui, T. Ueda, T. Oki, K. Sugita, N. Terahara et al. "α-Glucosidase inhibitory action of natural acylated anthocyanins. 1. Survey of natural pigments with potent inhibitory activity". J Agric Food Chem, vol. 49, pp. 1948-1951, Apr. 2001

[24] G.J. McDougall, F. Shpiro, P. Dobson, P. Smith, A. Blake et al. "Different polyphenolic components of soft fruits inhibit alpha-amylase and alpha-glucosidase" J Agric Food Chem, vol. 53, pp. 2760-6, Apr. 2005.

[25] A. Schäfer and P. Hőgger. "Oligomeric procyanidins of French maritime pine bark extract (Pycnogenol®) effectively inhibits α-glucosidase". Diabetes Research and Clinical Practice, vol. 77, pp. 41-6, Jul. 2007.

[26] M.S. Rabeta and N.R. Faraniza. "Total Phenolic Content and Ferric Reducing Antioxidant Power of The Leafss and Fruits of Garcinia Atrvirdis and Cynometra Cauliflora". International Food Research Journal, vol. 20, pp. 1691-1696, Feb. 2013

[27] M.N. Qaisar, B.A. Chaudhary, M.U. Sajid and N. Hussain. "Evaluation of α-Glucosidase Inhibitory Activity of Dichloromethane and Methanol Extracts of Croton bonplandianum Baill". Tropical Journal of Pharmaceutical Research, vol. 13, pp. 1833-1836, Nov. 2014.

[28] R.A. Kaskoos. "In-vitro α-glucosidase inhibition and antioxidant activity of methanolic extract of Centaurea calcitrapa from Iraq". American Journal of Essential Oils and Natural Products, vol. 1, pp. 122-125, Sept. 2013.

[29] A. Mun'im, Katrin, Azizahwati, A. Andriani, K.F. Mahmudah et al. "Screening of α-Glucosidase

inhibitory activity of Some Indonesian Medicinal plants". Int J Med Arom Plants, vol. 3, pp. 144-150, June 2013.

[30] T. Hertiani and I. Purwantini. "Anti Fungal Activity Of Essential Oil Distilled From Ethanol Extract Of Piper Betle L. Leaves Collected From Several Region In Yogyakarta, Againts Candida albicans". Indonesian Journal of Pharmacy, vol. 13, pp. 193-199, 2002.

[31] K. Alagesan, P. Thennarasu, V. Kumar, S. Sankarnarayanan and T. Balsamy. "Identification of α-Glucosidase Inhibitors From Psidium guajava Leafs and Syzygium cumini Linn. Seeds". International Journal of Pharma Sciences and Research, vol. 3, pp. 316-322, Feb. 2012.